



final report

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Biological Control of Bellyache Bush: host specificity testing of the *Jatropha* leaf-miner

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Executive summary

Introduced into northern Australia in the late 1800s as a hardy ornamental, bellyache bush (*Jatropha gossypifolia* L.) has become a serious weed of rangelands and riparian zones of northern Australia. It forms dense thickets, reducing the usefulness of land for pastoral and grazing purposes. Dense infestations along the Burdekin River in Queensland have reduced the carrying capacity to zero. All parts of the plants are toxic to stock.

Biological control is an important component of the long-term management strategy for bellyache bush in Australia. Bellyache bush has been a target for biological control since 1996 but there are currently no biological control agents established in Australia. As part of a renewed biological control effort, recent exploration was undertaken south of the equator in central South America with the aim of identifying new potential biocontrol agents. A yet to be described leaf-mining moth *Stomphastis* sp. (Lepidoptera: Gracillariidae) from Peru, was prioritised for further studies. The aim of this project was to import *Stomphastis* sp. into quarantine in Australia, establish a colony and complete host specificity testing of the agent. If suitably specific, an application to release the agent will be compiled and submitted to the necessary Australian Government regulatory bodies.

Over 200 pupae and over 200 larvae of the *Stomphastis* sp. were collected from 12 sites in northern Peru and were imported into the quarantine facility at the Ecosciences Precinct in Brisbane Australia in November 2014.

Adult *Stomphastis* sp. are small (less than 1 cm long) and live for an average of 10 days in the quarantine glasshouse. The majority of eggs are laid on the underside of leaves, usually next to a leaf vein. Newly emerged larvae mine directly into the leaf from the egg and remain in the leaf as they develop until pupation. Mature larvae exit the leaves and locate a suitable place to pupate. Most larvae pupate on the leaves but they may also pupate on other substrates. A generation from adult to adult takes 22 days under quarantine conditions.

No-choice host specificity testing of *Stomphastis* sp. has been completed for 40 test plant species, with at least five replications for each species. The agent laid eggs on numerous non-target species, however development of the agent only occurred on bellyache bush and its congener *Jatropha curcas*. When female *Stomphastis* sp. were provided with both bellyache bush and *J. curcas*, they oviposited equally on both species. Approximately 80% of eggs develop into adults on each of these species. *Jatropha curcas* is native to tropical America. It is a declared weed in Western Australia and the Northern Territory. It is also an approved target for biological control. As such a non-target risk assessment is not required.

Test results provide strong evidence that *Stomphastis* sp. is highly host specific and is suitable for release in Australia. It is expected that like other Gracillariidae, *Stomphastis* sp. will be an adept disperser, a desirable characteristic given the expansive areas across which bellyache bush occurs. A relatively short generation time and high fecundity also bodes well for its future as a biological control agent.

A release application, which will be submitted to the relevant regulatory bodies, has been drafted, as has a proposed release strategy.

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1 Background

Jatropha gossypifolia L. (Euphorbiaceae), commonly known as bellyache bush, has been widely dispersed throughout the world for ornamental and medicinal purposes and has become widely naturalised. Native to drier islands of the Caribbean and coastal Venezuela, bellyache bush is now found throughout tropical America from Florida in the north to Paraguay in the south as well as Hawaii. It is also found throughout tropical Africa, and tropical regions of Asia and the Pacific. Introduced into northern Australia in the late 1800s as a hardy ornamental, bellyache bush has become a serious weed of rangelands and riparian zones of northern Australia (Bebawi *et al.* 2007). It forms dense thickets, reducing the usefulness of land for pastoral and grazing purposes. Dense infestations along the Burdekin River in Queensland have reduced the carrying capacity to zero (Randall *et al.* 2009). All parts of the plants are toxic to stock, particularly seeds and deaths have been recorded during periods of drought when pasture is scarce (DEEDI 2009). Monotypic stands suppress seedling recruitment of native species, reducing biodiversity and impacting fire regimes due to reduced fuel load. The shallow root system causes increases erosion along creek and river banks (Csurhes 1999, Bebawi *et al.* 2007). Bellyache bush is also highly toxic to humans. As little as three seeds can kill a small child and the sap can cause severe skin irritation.

Bellyache bush is widespread across northern Australia, particularly along watercourses and connected floodplains (DEEDI 2009). In Queensland the most extensive infestations occur within the Burdekin River catchment and tributaries, as well as the Fitzroy, Walsh, Palmer, Flinders and Gregory River catchments and the headwaters of Lake Eyre. In the Northern Territory widespread infestations occur in the Daly, McArthur, Roper and Victoria River catchments, as well as the Gulf region and the Barkly Tableland. In Western Australia bellyache bush is prevalent in the East Kimberley with smaller infestations in the West Kimberley and the Pilbara. Six morphological and phenological distinct populations occur in Australia: Queensland Bronze, Queensland Green and Queensland Purple in Queensland, Darwin Purple and Katherine Purple in the Northern Territory and Kununurra Green in Western Australia (Bebawi *et al.* 2007).

Biological control is an important component of the long-term management strategy for bellyache bush in Australia. Bellyache bush has been a target for biological control since 1996. Native range surveys in Mexico, northern South America, and the Caribbean resulted in the release of one agent, the seed feeding jewel bug *Agonosoma trilineatum* (F.) (Heteroptera: Scutelleridae), which failed to establish (Heard *et al.* 2012). In addition, host specificity testing of the leaf rust *Phakopsora arthuriana* Buriticá & J.F. Hennen (Pucciniales: Phakopsoraceae) (synonym *Phakopsora jatrophiicola* Cummins) was initiated (Heard *et al.* 2012). Testing of this rust has finished and final biological studies are currently being completed.

As part of a renewed biological control effort, recent exploration was undertaken south of the equator in central South America with the aim of identifying new potential biocontrol agents. Field surveys in 2012 and 2013 identified 11 insect species, one mite species and a leaf rust (Dhileepan *et al.* 2014). Of these species, a yet to be described leaf-mining moth *Stomphastis* sp. (Lepidoptera: Gracillariidae) from Peru, was prioritised for further studies. The aim of this project was to import *Stomphastis* sp. into quarantine in Australia, establish a colony and complete host specificity testing of the agent. If suitably specific an application to release the agent will be compiled and submitted to the necessary federal regulatory bodies.

2 Project objectives

2.1 *Jatropha* leaf miner established in quarantine (MET).

A colony of the leaf-miner has been successfully established and maintained in quarantine since November 2014.

2.2 Life cycle studies for the *Jatropha* leaf-miner completed (MET).

Under quarantine glasshouse conditions adult *Stomphastis* sp. live for an average of 10 days (up to 28 days). Females lay an average of 101 eggs, mostly on the underside of leaves near a leaf vein. Newly emerged larvae mine directly into the leaf from the egg and remain in the leaf as they develop until pupation. Development from egg to adult takes an average of 22 days.

2.3 Complete host specificity testing of 40 test plant species (MET).

No-choice host specificity testing of *Stomphastis* sp. has been completed for 40 test plant species, with at least five replications for each species.

2.4 If oviposition and larval development occurred on any non-target test plants, then choice oviposition and larval development tests completed and a non-target risk assessment developed (MET).

During host specificity testing oviposition and complete larval development only occurred on bellyache bush and *Jatropha curcas*. *Jatropha curcas* is an approved target for weed biological control, so while it is not the focus weed of this work, it is not a non-target species. The comparison trial we conducted with *J. curcas* and bellyache bush was to explore the suitability of it as an alternate host. When female *Stomphastis* sp. are provided with both bellyache bush and *J. curcas*, they oviposited equally on both species. Approximately 80% of eggs develop into adults on each of these species.

It is highly likely that *J. curcas* will be attacked by *Stomphastis* sp. nova once it has been released in Australia. Given the anticipated good dispersal ability of the moth, attack is unlikely to be limited to *J. curcas* infestation occurring sympatrically with bellyache bush. *Stomphastis* sp. nova larvae reduce the photosynthetic area of attacked leaves, which could impact the growth and reproductive ability of *J. curcas* plants.

Jatropha curcas is native to tropical America. Scattered naturalised populations occur in Queensland and the Northern Territory and it is a declared weed in Western Australia and the Northern Territory. Though not declared as a weed, *J. curcas* is regarded as an invasive in Queensland as well. Any potential attack of *J. curcas* can thus be considered beneficial. Unlike the bellyache bush which sheds its leaves during winter (cooler or drier months), the *J. curcas* trees do not shed their leaves, and hence could be a vital alternative host in the absence of bellyache bush leaves. This would be a very important benefit for the bellyache bush biocontrol.

2.5 Submit application for field release (if proven host-specific) (PARTIALLY MET).

Host specificity testing of the required 40 species has been completed, thus satisfying milestone 2.3. Stakeholders in the Northern Territory have shown much interest in this agent and hence it is important to test some native Euphorbiaceae species common in the Top End. This will add to the robustness of the application and thus reduce potential delays with the approval process at a federal level. This may also facilitate the approval to release the insect in the Northern Territory (the agent when approved will be field released in Queensland, the Northern Territory and Western Australian [WA]). A draft of the application is attached. The application will be finalised once the additional species has been tested. During this time we will develop a Climex model for the moth which will identify the areas climatically suitable for successful establishment of the moth. These test plant species have sourced from the Northern Territory and we are waiting for them to reach a size suitable for testing.

2.6 If testing supports, provision of draft release strategy aimed at optimising geographic spread of the agent and sufficient insect numbers at each site to assist establishment (MET).

A proposed release strategy has been drafted. The insect is currently contained within our quarantine facility at the Ecosciences Precinct in Brisbane and it is from here that the insect will be initially mass reared, until a colony is established at the Queensland Government research facility at Charters Towers (TWRC). TWRC will be the primary mass rearing facility for this insect in Queensland and the release effort will be focussed in areas with major bellyache bush infestations. The Northern Territory Government has expressed interest in mass rearing and releasing at their Darwin research facility. We will supply the agents to stakeholders in WA (in partnership with the Department of Agriculture and Food) if interest is expressed. Given the ease with which the insect can be reared it will be valuable to involve organisations such as local councils and catchment groups with the mass rearing and release program.

3 Methodology

3.1 Source of insects and rearing

Stomphastis sp. was commonly observed on *J. gossypiifolia* in the San Martín province in northern Peru and occasionally on *J. curcas* L. (Dhileepan *et al.* 2014). In the Manuel María Caballero province of central Bolivia the leaf miner was commonly observed on *J. gossypiifolia* and *J. excisa* Griseb and rarely on *J. curcas* and *J. clavuligera* Müll.Arg. This is the first *Stomphastis* species to be documented in South America. Originally thought to be *Stomphastis thraustica* Meyrick, a well-known pest of *J. gossypiifolia* and *J. curcas* in Asia and Africa, it is currently being described as a new species by Dr Jurate De Prins (Royal Belgian Institute of Natural Sciences).

The genus *Stomphastis* is a member of the Gracillariidae, a family of leaf mining microlepidopterans. The genus *Stomphastis* contains 17 species; all from Africa and/or Asia (De Prins and De Prins 2013). Two species are known pests: *S. thraustica* and *S. chalybacma* Meyrick (a pest of *Caesalpinia decapetala* (Roth) Alston, *C. pulcherrima* (L.) Sw. and *Albizia saman* F. Muell. in India; NBAII 2013).

Over 200 pupae and over 200 larvae of the *Stomphastis* sp. were collected on *J. gossypiifolia* from 12 sites in northern Peru and were imported into our quarantine facility at the Ecosciences Precinct in

Australia in November 2014. A colony was established on potted bellyache bush plants in gauze covered cages (100 x 90 x 45 cm) kept in a heated glasshouse maintained at 30°C and 60%RH and natural daylight. Each week, between 40 and 60 adults were released into a gauze covered cage (100 x 90 x 45 cm) containing eight to ten bellyache bush plants (depending on size). Surviving adults were collected after several days and placed into a freezer. Leaves containing pupated individuals were collected and placed into a sealed 30 x 20 x 10 cm décor® container with a gauze window in the lid for ventilation.

3.2 Source of plants and propagation

Bellyache bush plants used for rearing and testing of *Stomphastis* sp. were propagated by either seed, field collected seedlings or cuttings. All leaves were removed from field collected material to minimise the risk of introducing unwanted phytophagous insects (such as the native gracillariid known to attack bellyache bush in Australia (*Epicephala* sp.; Wilson 1997). Due to the ease with which bellyache bush establishes from cuttings, cuttings were also frequently taken from our larger potted plants. Plants from all six populations known to occur in Australia were kept for testing, however the majority of plants were either Queensland Bronze, Katherine Green or Kununurra Green. Commercial potting mix was used in various sized round plastic pots (most 14-20 cm diameter). Slow release fertilizer was added in early spring, summer and autumn. During the warmer months, most of the bellyache bush plants were kept in a shade house (30 or 60% shade) on the roof of the Ecosciences Precinct building, where they were watered three times daily, plus rainfall. During the cooler months bellyache bush lose their leaves in Brisbane so the majority of plants were moved to a heated glasshouse maintained at 30°C during this period with watering three times daily.

Test plants were sourced from local nurseries or (where this was not possible) were grown from cuttings collected in the field. *Jatropha curcas* and *Ricinus communis* L. were grown from field collected seed. Plants have also recently been sourced from the Northern Territory. These Euphorbiaceae (and related families) species are common in the Top End. Given that the agent will likely be released in the Northern Territory, including these species will improve the robustness of the test list.

3.3 Temperature trials

Temperature trials are currently in progress; trials are running at 15°C, 17.5°C, 30°C and 35°C. Additional temperatures will be added to the trial. Temperature control cabinets are set to 12 hour light per day. As humidity could not be controlled a container of water was placed in each cabinet. To determine adult survival, 20 newly emerged adults were added to a one litre rectangular takeaway food container with a gauze covered lid, containing a moistened sheet of paper towel and small container of water with a dental wick protruding from the lid. Containers were checked daily and dead adults removed. A minimum of six replicates were completed per temperature.

To determine larval survival adult moths were released into a small cage containing a single potted bellyache bush plant for one night. After one night adults were removed and eggs marked on the plant. Plants were checked daily for larval emergence and then twice a week until adult emergence or until no more larval development was detected. A minimum of six replicates were completed per temperature.

3.4 Test list

Jatropha is in the Jatropheae tribe in the Crotonoideae subfamily of Euphorbiaceae. It is the only representative of the tribe Jatropheae in Australia. The genus contains approximately 170 species, of which a few are cultivated, mostly for ornamental purposes. *Jatropha curcas* is promoted in some countries as a biofuel. In addition to *J. gossypifolia*, *J. curcas*, *J. podagrica* and *J. multifida* are also known to be cultivated as ornamentals in Australia. Naturalised populations of *J. curcas* occur in

northern Australia. It is a declared species in the Northern Territory and Western Australia and it has been approved as a target for weed biological control.

The family Euphorbiaceae is in the order Malphigiales (APG III 2009). Euphorbiaceae Jussieu *sensu stricto* is represented in Australia by approximately 38 genera (31 native) and 288 species (241 native). Groupings within the family are under review. Three of five subfamilies are represented in Australia: Crotonoideae, Acalyphoideae, and Euphorbioideae. The two most closely related families, Peraceae and Rafflesiaceae, are not present in Australia (the subfamily Cheilosoideae is also absent from Australia).

The host test list of 40 species, was based on the list approved for *Agonosoma trilineatum* (Heard *et al.* 2009), which was developed following the phylogenetic centrifugal method (Wapshere 1974, 1989), focusing on phylogenetically-related native species occurring in northern Australia where bush bellyache is invasive. Since the testing of *A. trilineatum*, Euphorbiaceae has been split into four families; Euphorbiaceae Jussieu *sensu stricto*, Phyllanthaceae, Picrondendraceae and Putranjivaceae (Stevens 2001–). Putranjivaceae is no longer considered to be closely associated to the Euphorbiaceae family; one representative of this family was included as an outlier. Species included in the test list approved for *A. trilineatum* but omitted from this proposed list were either difficult to source or were replaced by species deemed more suitable. Species from unrelated families were also removed.

3.5 Host specificity testing

Host specificity testing was conducted from December 2014 until April 2017 in a quarantine glasshouse maintained at 30°C and 60% RH.

3.5.1 No-choice tests

All plants in the test list were subjected to no-choice tests. Twenty newly emerged *Stomphastis* sp. adults were released into a 45 x 45 x 90 cm gauze covered cage containing a single potted test plant and a small sealed container of an isotonic fluid (such as Gatorade) with a dental wick protruding from the lid (to provide the moths with sustenance). With each round of testing, at least one bellyache bush plant was also included as control, however only ten newly emerged adults were released into this cage. Plants were checked after seven days for eggs and larval mines (which were counted) and again when all adults had died. Any test plants with eggs and or larval mines were monitored until adults had emerged on the bellyache bush test plant. Each test species was subjected to a minimum of five replicates.

3.5.2 Choice comparison trial

Any test plants on which larval development occurred were subjected to a comparison trial with the target. Ten newly emerged *Stomphastis* sp. adults were released into a 100 x 45 x 90 cm gauze covered cage containing a single potted *J. curcas* plant (the only species other than bellyache bush on which larvae completed development) and one bellyache bush plant (arranged so that no plants were touching). Adults were removed from the cage after one day and the number of eggs on each plant counted. Plants were monitored and the larvae, pupae and adults and the duration of each life stage was recorded. Similar sized plants were chosen for each replicate and eight replications were completed.

3.5.3 Choice oviposition trials

Since female *Stomphastis* sp. oviposited on many non-target species under no-choice conditions, multiple choice trials were conducted to examine if they would be oviposited on in the presence of the target. Ten newly emerged *Stomphastis* sp. adults were released into a 100 x 45 x 90 cm gauze covered cage containing a single potted plant each of a selected number of native species on which eggs were consistently laid (*Croton verreauxii*, *Baloghia inophylla*, and *Aleurites moluccana*) and a single bellyache bush plant (arranged so that no plants were touching). Plants were checked every three days and the number of eggs counted until all adults died. Plants were monitored until adults emerged on the bellyache bush plant tested. Similar sized plants were chosen for each replicate. Plant availability limited the trial to three replications.

4 Results

4.1 Life history

Adult *Stomphastis* sp. are small (less than 1 cm long; Fig. 1) and live for an average of 10 days in the quarantine glasshouse (15 days if provided with isotonic fluid such as Gatorade). They emerge during the evening/early morning and mate during the early morning. Females do not generally lay any eggs on the day they emerge. They lay an average of 101 eggs (up to 172 eggs). Eggs are tiny and are barely visible to the naked eye (Fig. 1). The majority of eggs are laid on the underside of leaves, usually next to a leaf vein. Some eggs are also laid on upper side of leaves. Egg development takes an average of four days (Table 1). Newly emerged larvae mine directly into the leaf from the egg and remain in the leaf as they develop until pupation (Fig. 2). Mature larvae exit the leaves and locate a suitable place to pupate. Most larvae pupate on the leaves but they may also pupate on other substrates such as bellyache bush branches and cage walls; they either crawl off the leaf or drop from a spun thread. Occasionally larvae may pupate within the leaf. Pupation takes an average of eight days (Table 1). A generation from adult to adult takes 22 days under quarantine conditions (Table 1).

Table 1. Life stage data for *Stomphastis* sp.

Life stage	Mean	Range
Adult survival (with isotonic fluid)	9.9 days (14.6 days)	up to 21 days (up to 28 days)
Egg development time	4.1 days	3-6 days
Larval development time	14.7 days	12-19 days
Pupal development time	8.0 days	7-9 days

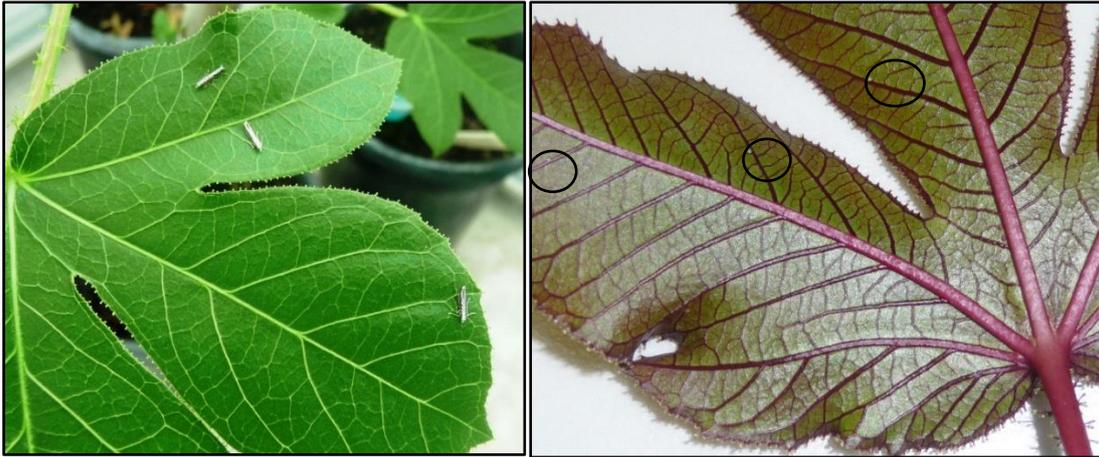


Fig. 1. Adult *Stomphastis* sp. (left) and *Stomphastis* sp. eggs (right) on bellyache bush leaves.

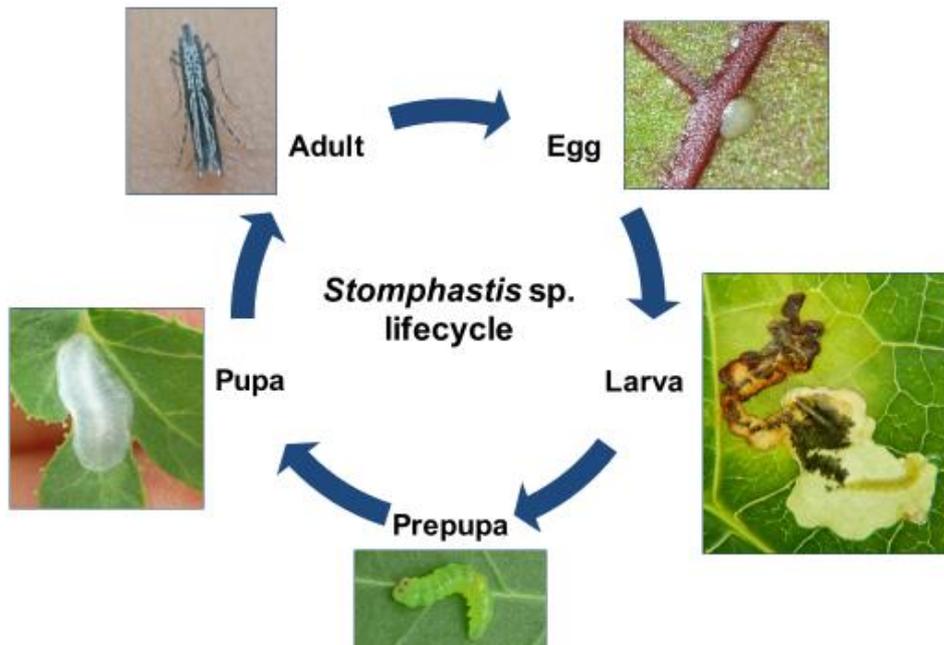


Fig. 2. *Stomphastis* sp. lifecycle.

In temperature trials to date, adults have survived up to 25 days at 15°C and up to six days at 35°C (Fig. 3). Egg lay has occurred at all temperatures tested (15°C to 35°C) but larval development through to adult has only occurred from 17.5°C through to 35°C at this stage (Table 2). The shortest generation time from egg to adult has been at 30°C (minimum of 13 days). At 17.5°C a generation has taken up to 47 days. Studies are ongoing.

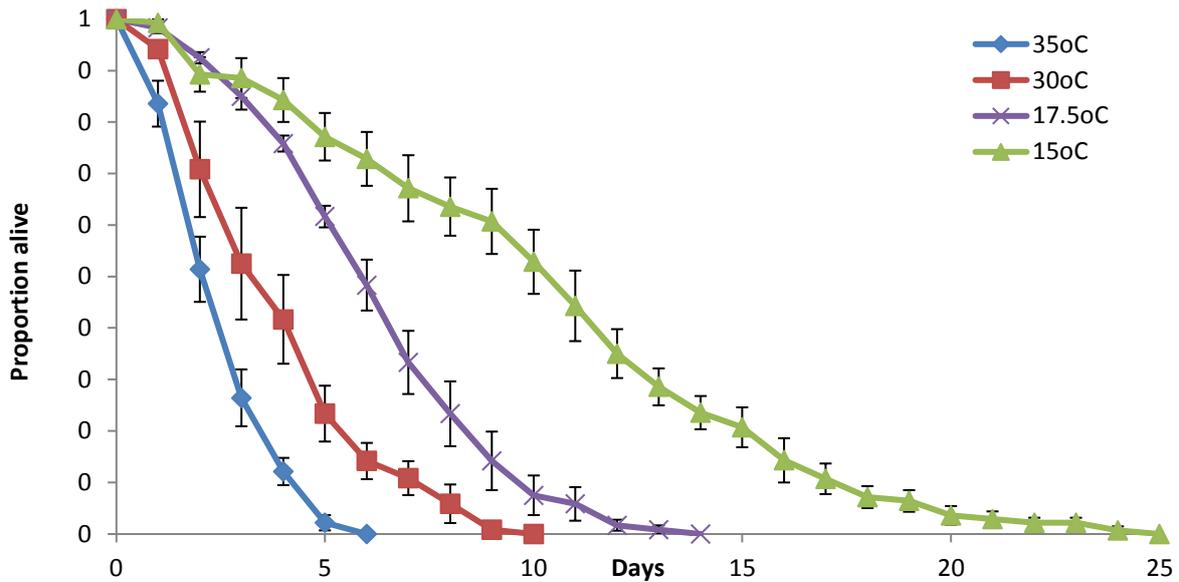


Fig. 3. Adult survival at constant temperatures.

Table 2. *Stomphastis* sp. development at constant temperatures (in progress).

	15°C	17.5°C	30°C	35°C
Days to egg hatch	13.3 (range 11-14)	5.3 (range 4-8)	2.4 (range 2-4)	3.1 (range 2-4)
% egg hatch (mean ± SE)	0.71 ± 0.08	0.94 ± 0.01	0.97 ± 0.01	0.87 ± 0.03
Days from egg to adult	-	29.3 (range 26-47)	14.2 (range 13-18)	22.8 (range 20-25)
% egg to adult (mean ± SE)			0.45 ± 0.09	

4.2 Host specificity testing

4.2.1 No-choice tests

Under no-choice conditions *Stomphastis* sp. females laid eggs on 24 of the 40 test species (Table 3). Egg hatch occurred on 19 of these species. However in all cases, except *J. curcas*, the 1st instar larvae died shortly after emerging; this includes congeners *J. multifida* and *J. podagrica*. The larval mines on these species ranged in size from 1 to 20 mm in length per mine (Fig. 4). Larval development was only observed on bellyache bush and *J. curcas* plants. The number of adults that emerged from *J. gossypifolia* plants during no-choice trials ranged from 21 to 193 (mean 83.5). An average of 51 adults emerged from *J. curcas* (Table 3; Fig. 5).

Table 3. No-choice host specificity test results.

Test plants	Status [#]	Reps	Eggs	1st instar larvae	Larval development	Adults*
Euphorbiaceae						
Crotonoideae						
Jatrophaeae						

<i>Jatropha gossypifolia</i> L.	Target	36	Y	Y	Y	83.5 (8.7)
<i>Jatropha curcas</i> L.	I	5	Y	Y	Y	51.4 (29.1)
<i>Jatropha podagrica</i> Hook.	O	5	Y	Y	N	N
<i>Jatropha multifida</i> L.	O	5	Y	Y	N	N
Aleuritideae						
<i>Aleurites</i> sp.	N	5	Y	Y	N	N
Codiaeae						
<i>Baloghia inophylla</i> (G.Forst.) P.S.Green	N	5	Y	Y	N	N
<i>Codiaeum variegatum</i> (L.) A.Juss.	E	5	N	N	N	N
Crotonoideae						
<i>Croton acronychioides</i> F.Muell.	N	7	Y	N	N	N
<i>Croton insularis</i> Baill.	N	5	Y	N	N	N
<i>Croton verreauxii</i> Baill.	N	5	Y	Y	N	N
Manihoteae						
<i>Manihot esculentum</i> Crantz	C	6	N	N	N	N
<i>Manihot grahamii</i> Hook.	I	6	Y	Y	N	N
Ricinocarpeae						
<i>Beyeria lechenaultii</i> (DC.) Baill.	N	6	Y	N	N	N
<i>Beyeria viscosa</i> (Labill.) Miq.	N	5	N	N	N	N
<i>Ricinocarpos pinifolius</i> Desf.	N	3	Y	N	N	N
Acalyphoideae						
Acalypheae						
<i>Macaranga tanarius</i> (L.) Müll.Arg.	N	5	N	N	N	N
<i>Mallotus philippensis</i> (Lam.) Muell.Arg.	N	5	N	N	N	N
<i>Ricinus communis</i> L.	I	5	Y	Y	N	N
Alchorneae						
<i>Alchornea ilicifolia</i> (J.Sm.) Muell.Arg.	N	5	Y	Y	N	N
Adenoclineae						
<i>Endopermum</i> sp.	N	5	Y	Y	N	N
Omphaleae						
<i>Omphalea celata</i> P.I.Forst	N	6	Y	Y	N	N
Euphorbioideae						
Euphorbieae						
<i>Euphorbia grantii</i> Oliv.	E	5	Y	Y	N	N
<i>Euphorbia pulcherrima</i> Willd. ex Klotzsch	E	5	N	N	N	N
<i>Euphorbia tithymaloides</i> L.	E	5	N	N	N	N
Hippomaneae						
<i>Homalanthus populifolius</i> Graham	N	5	N	N	N	N
<i>Microstachys chamaelea</i> (L.) Hook.f.	N	5	Y	Y	N	N
Phyllanthaceae						
Antidesmatoideae						
<i>Antidesma bunius</i> (L.) Spreng.	N	5	Y	Y	N	N
<i>Antidesma ghaesembilla</i> Gaertn.	N	5	Y	Y	N	N
Phyllanthoideae						
<i>Actephila lindleyi</i> (Steud.) Airy Shaw	N	5	Y	Y	N	N
<i>Breynia cernua</i> (Poir.) Mull.Arg.	N	5	Y	Y	N	N

<i>Breynia oblongifolia</i> (Mull.Arg.) Mull.Arg	N	3	Y	Y	N	N
<i>Bridelia exaltata</i> F. Muell.	N	6	Y	N	N	N
<i>Cleistanthus hylandii</i> Airy Shaw	N	6	N	N	N	N
<i>Flueggea virosa</i> (Willd.) Voigt	N	5	Y	N	N	N
<i>Glochidion ferdinandi</i> (Muell.Arg.) F.M.Bailey	N	5	N	N	N	N
<i>Phyllanthus cuscutiflorus</i> S.Moore	N	5	N	N	N	N
Picrodendraceae						
<i>Austrobuxus swainii</i> (Beuzev. & C.T.White) Airy Shaw	N	5	N	N	N	N
<i>Dissiliaria baloghoides</i> F.Muell. ex Baill.	N	5	N	N	N	N
<i>Petalostigma pubescens</i> Domin	N	5	N	N	N	N
<i>Sankowskyia stipularis</i> P.I.Forst.	N	5	N	N	N	N
Putranjivaceae						
<i>Drypetes deplanchei</i> (Brongn. & Gris) Merr.	N	5	Y	Y	N	N

#Status: N=ative, O=ornamental, C=crop, I=invasive. *Mean (standard error)



Fig. 4. 1st instar larval mines of *Stomphastis* sp. on *Croton verreauxii* (left) and *Baloghia inophylla* (right).



Fig. 5. *Stomphastis* sp. damage to bellyache bush (left) and *J. curcas* (right).

4.2.2 Choice comparison trial

Paired-choice trials have been completed for *J. curcas*, the only non-target species on which *Stomphastis* sp. has completed development during no-choice host specificity trials. When female *Stomphastis* sp. are provided with both bellyache bush and *J. curcas*, they oviposited equally on both species; in nine replicates 51% of eggs were laid on *J. curcas* (Fig. 6). Approximately 80% of eggs develop into adults on each of these species (Fig. 7).

4.2.3 Choice oviposition trials

In choice oviposition trials, an average of 92.8% of eggs were laid on bellyache bush; significantly greater than the percentage laid on the non-target species (Fig. 8). No larval development occurred on any species other than bellyache bush, confirming results from no-choice trials.

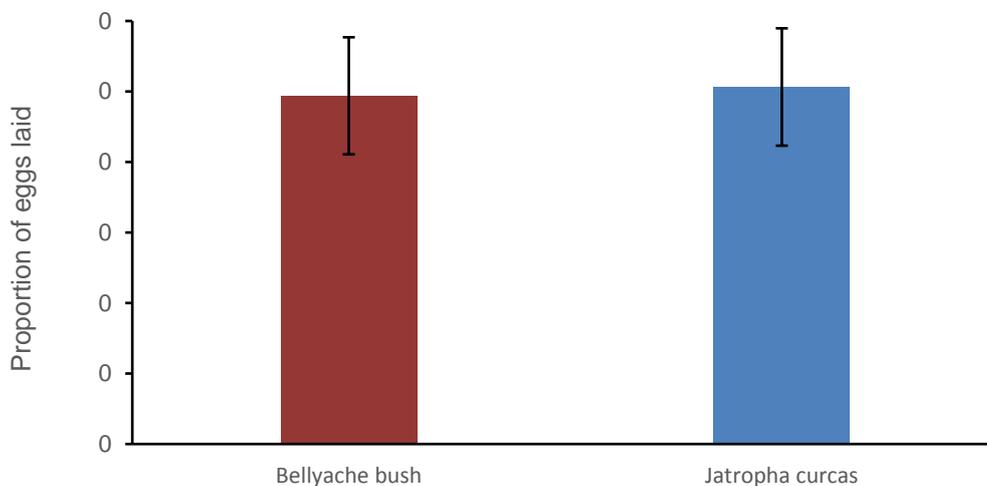


Fig. 6. Proportion of *Stomphastis* sp. eggs laid on bellyache bush and *Jatropha curcas* in paired choice trials.

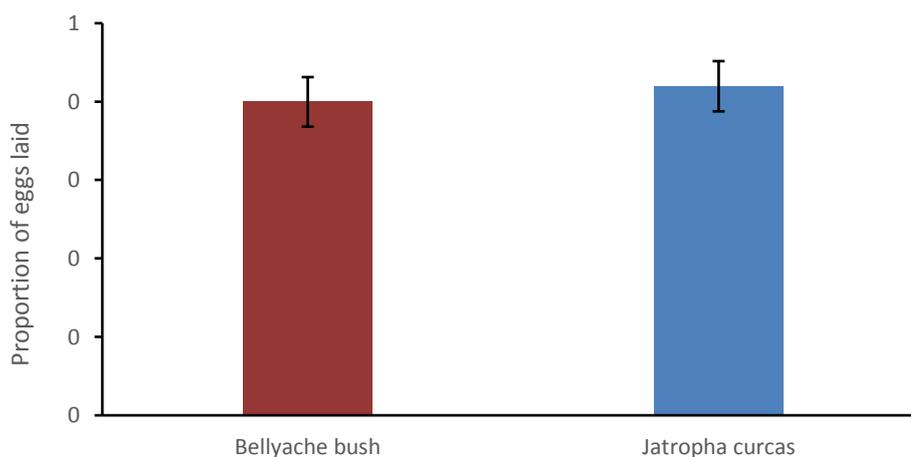


Fig. 7. Proportion of *Stomphastis* sp. eggs laid that completed development into adults on bellyache bush and *Jatropha curcas* in paired choice trials.

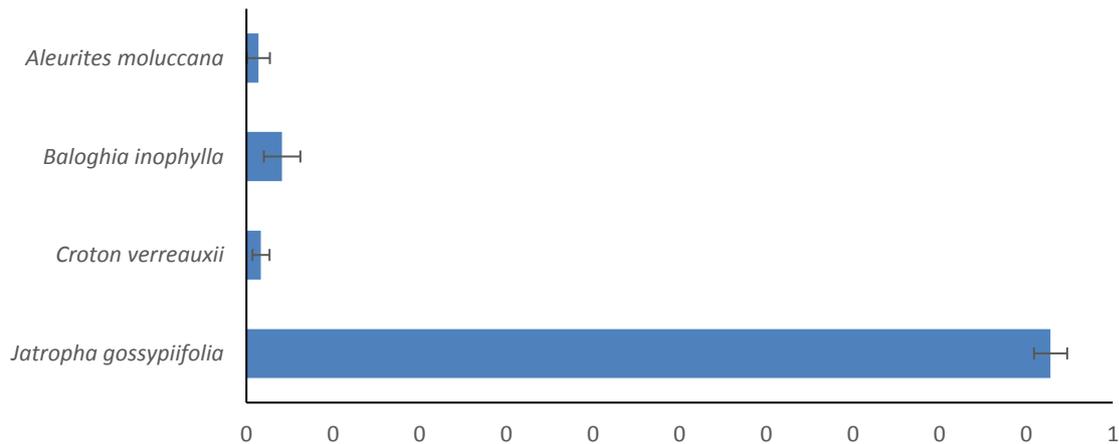


Fig. 8. Proportion of eggs laid on bellyache bush and three non-target species under choice conditions.

5 Discussion

No-choice host specificity testing of *Stomphastis* sp. has been completed with 40 plant species tested, and although eggs have been laid on many of these species, larval development only occurred on bellyache bush and *Jatropha curcas* (also an approved target for biological control). *Jatropha curcas* is native to tropical America. Scattered naturalised populations occur in Queensland and the Northern Territory and it is a declared weed in Western Australia and the Northern Territory. Though not declared as a weed, *J. curcas* is regarded as an invasive in Queensland as well. It is also an approved target for biological control.

These test results provide strong evidence that *Stomphastis* sp. is highly host specific and is suitable for release in Australia. Several leaf mining Gracillariidae moths have been utilised for weed biological control in Australia; *Dialectica scariella* against *Echium plantagineum*, *Cuphodea profluens* against prickly acacia and *Neurostrotia gunniella* against *Mimosa pigra*. Released in the Top End of Australia, *N. gunniella* dispersed rapidly following release, and is now present wherever *M. pigra* occurs in the Northern Territory (Wilson and Flanagan 1990; Wilson and Forno 1995). It is expected that *Stomphastis* sp. will also be an adept disperser, a desirable characteristic given the expansive areas across which bellyache bush occurs. A relatively short generation time and high fecundity also bodes well for its future as a biological control agent.

5.1 Draft release strategy

Stomphastis sp. nova is an easy insect to rear. Rearing will require bellyache bush plants with ample leaf material and cages of some description to contain the insects with the plants. The insect has a short development time (a generation can be completed in less than a month), so under ideal conditions populations should build up rapidly.

Stomphastis sp. nova is currently contained within our quarantine facility at the Ecosciences Precinct in Brisbane (1 – Fig. 9) and it is from here that the insect will be initially mass reared, until a colony is established at the Queensland Government research facility at Charters Towers in north Queensland (Tropical Weed Research Centre – TWRC). A limited number of releases will be made from Brisbane

to suitable infestations within a day's drive of Brisbane. TWRC will be the primary mass rearing facility for this insect in Queensland (2 – Fig. 9). The release effort will be focussed in areas with major bellyache bush infestations, along the Burdekin River from Charters Towers to Home Hill, Hughenden, Gulf of Carpentaria, along the Gregory River, Normanton, and along the Palmer River in Cape York. There will be an opportunity to explore partnership with various community and natural resource management (NRM) groups (e.g. Mitchel River Catchment Group, Cape York indigenous groups, Desert Channels, etc.) in the rearing and release of the leaf-miner.

The Weed Management Branch of the Northern Territory Government's Department of Environment and Natural Resources have expressed interest in mass rearing and releasing at their Darwin research facility (3 – Fig. 9). We will supply the agents to stakeholders in WA (in partnership with Dept. of Agriculture and Food, Western Australia), for rearing and field release there if interest is expressed. Given the ease with which the insect can be reared it will be valuable to involve organisations such as local councils and catchment groups with the mass rearing and release program. This will necessarily involve workshops with relevant parties. The insect may be sent to some community groups/property managers via post. Pupae will be the best life stage to be transported via this method. Obviously the extent of the release program involving community and NRM groups will depend on funding.

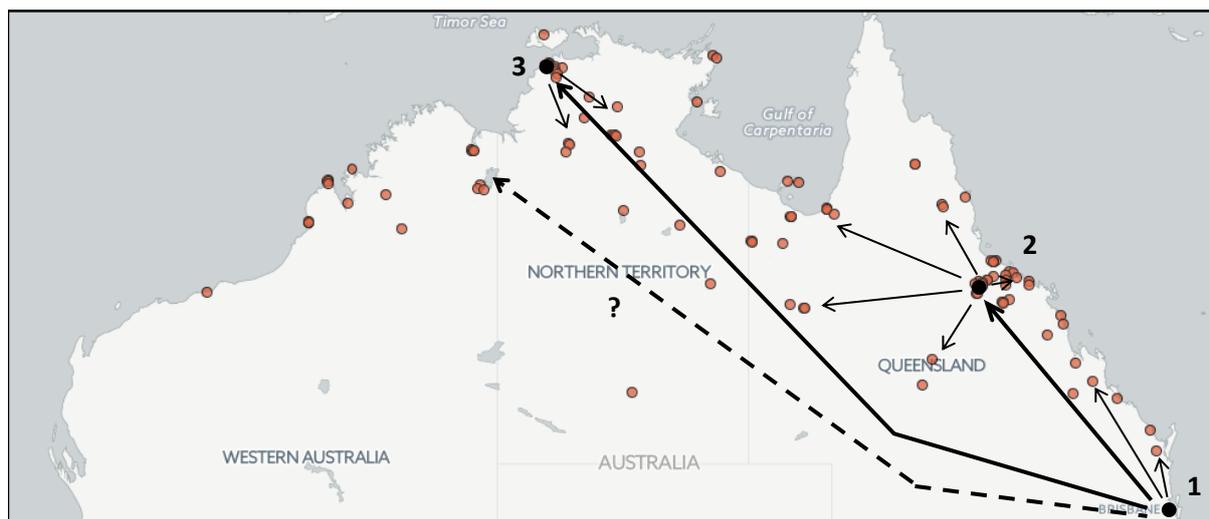


Fig. 9. Schematic representation of the proposed release strategy for *Stomphastis* sp. on a map displaying bellyache bush herbarium records.

At this stage we are unsure as to the best method for releases, but they will likely involve a combination of adults and pupae. Pupae will be the easiest method, particular where multiple days travel to release sites are required. Pupation takes five to seven days. Pupae that are several days old can be cut from leaves or whole branches can be cut from an infested plant. The cut branches can be hung in bellyache bush plants in the field or a container with the pupae can be strategically located. Adults can be released at field sites located closer to the mass rearing facility. Conditions in quarantine are very different to the field so it is difficult to provide an optimal number of individuals for a successful release. This is something that we will ascertain early in the release program. A higher number of individuals means a greater level of genetic diversity, which is desired. Initial release numbers will range from 100-1000 individuals per release, until the optimal number for establishment is determined.

6 Conclusions/recommendations

Quarantine test results provide strong evidence that *Stomphastis* sp. is highly host specific and is suitable for release in Australia. Once approval has been received from regulatory bodies for the agent's release, we recommend that the agent is mass-reared and field released in Queensland from the Tropical Weeds Research Centre in Charters Towers. Various Landcare and community groups in Queensland, the Northern Territory and Western Australia will be trained in the rearing and release methods of the *Jatropha* leaf-miner. Starter colonies of the agent will be supplied to various community groups in Queensland, the Northern Territory and Western Australia for mass-rearing and field release of the agent. The release sites will be monitored regularly for evidence of field establishment, spread and damage levels.

7 Key messages

- The *Jatropha* leaf-miner *Stomphastis* sp. is highly host specific and is suitable for release in Australia.
- A relatively short generation time and high fecundity also bodes well for *Stomphastis* sp. for its use as a biological control agent in Australia.
- It is expected that *Stomphastis* sp. will also be an adept disperser, a desirable characteristic given the expansive areas across which bellyache bush occurs.
- A release application will be submitted to the relevant regulatory bodies.
- If approved, the *Jatropha* leaf-miner is the first leaf-feeding insect to be released against bellyache bush in Australia.

8 Bibliography

- Bebawi F.F., Vitelli J.S., Campbell S.D., Vogler W.D., Lockett C.J., Grace B.S., Lukitsch B. and Heard T.A. 2007. The biology of Australian weeds 47. *Jatropha gossypifolia* L. *Plant Protection Quarterly* **22**: 42-00.
- Csurhes S.M. 1999. Bellyache bush (*Jatropha gossypifolia*) in Queensland. Pest Review Series – Land Protection. Queensland Government Department of Natural Resources.
- De Prins J & De Prins W 2013. Global Taxonomic Database of Gracillariidae (Lepidoptera). Available at: <http://www.gracillariidae.net> [Accessed 1st October 2013].
- Dhileepan, K., Nesar, S. and De Prins, J. 2014. Biological control of bellyache bush (*Jatropha gossypifolia*) in Australia: South America as a possible source of natural enemies. In *Proceedings of the XIV International Symposium on Biological Control of Weeds*, eds F.A.C. Impson, C.A. Kleinjan, and J.H. Hoffmann, pp. 5-10 (Kruger National Park, South Africa)
- Heard, T.A., Dhileepan, K., Bebawi, F., Bell, K. and Segura, R. 2012. *Jatropha gossypifolia* L. – bellyache bush. In *Biological control of weeds in Australia: 1960 to 2010*, eds M. Julien, R.E. McFadyen and J. Cullen, pp. 324-33. (CSIRO Publishing, Melbourne)
- Heard, T.A., Chan, R.R., Senaratne, W.K.A.D., Palmer, W.A., Lockett, C. and Lukitsch, B. 2009. *Agonosoma trilineatum* (Heteroptera: Scutelleridae) a biological control agent of the weed bellyache bush, *Jatropha gossypifolia* (Euphorbiaceae). *Biological Control* **48** (2), 196-203.
- NBAII 2013. National Bureau of Agriculturally Important Insects: *Chilocorus nigrita* (Fabricius). Available at: <http://nbaii.res.in/Featured%20insects/Chilocorus.htm> [Accessed 1st October 2013].
- Stevens, P.F. 2001– . Angiosperm Phylogeny Website. Version 12, July 2012. Available at: <http://www.mobot.org/MOBOT/research/APweb/> [Accessed 6th June 2016]
- Wapshere, A.J., 1974. A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* **77**, 210–211.
- Wapshere, A.J., 1989. A trial sequence for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology* **114**, 515–526
- Wilson, C.G. 1997. Phytophagous insect fauna of two weeds, *Hyptis suaveolens* (L.) Poit. and *Jatropha gossypifolia* L., in Australia's Northern Territory. *Australian Entomologist* **24**, 55–60.
- Wilson C.G., Flanagan G.J. 1990. Establishment and spread of *Neurostrota gunniella* on *Mimosa pigra* in the Northern Territory. In: Swarbrick JT (ed) *Proceedings of the Ninth Australian Weeds Conference*. Australian Weed Science Society, pp 505–507
- Wilson C.G. Forno I.W. 1995. The biological control program against *Mimosa pigra* in Australia's Northern Territory. In: *Proceedings of the eight International Symposium on Biological Control of Weeds*. Christchurch, New Zealand, pp 75–80.

9 Appendices

1. Taylor, D.B.J. and Dhileepan, K. 2017. Host specificity testing of *Stomphastis* sp. nova, an agent for the biological control of *Jatropha gossypifolia*. Draft report to be submitted in support of an application to release the agent from quarantine. Biosecurity Queensland, Department of Agriculture and Fisheries (**Appendix 1**).
2. Taylor, D.B.J. and Dhileepan, K. 2016. Prospects for the biological control of *Jatropha gossypifolia*: *Stomphastis* sp. as a potential agent from South America, pp. 233-236. In: Randall, R., Lloyd, and S., Borger, C. (eds), *Proceedings of the 20th Australasian Weeds Conference*, Weeds Society of Western Australia, Perth, 11-15 September 2016 (**Appendix 2**).
3. Taylor, D.B.J. and Dhileepan, K. 2015. Prospects for the biological control of bellyache bush, pp. 71-74. In: W. Vogler (ed.), *Proceedings of the 13th Queensland Weed Symposium*, The Weed Society of Queensland, Longreach, Queensland, 14-17 September 2015 (**Appendix 3**).
4. Dhileepan, K., Nesar, S. and J. De Prins. 2014. Biological control of bellyache bush (*Jatropha gossypifolia*) in Australia: South America as a possible source of natural enemies, pp. 5-10. In: Impson, F.A.C., Kleinjan, C.A., and Hoffmann, J.H. (eds), *Proceedings of the XIV International Symposium on Biological Control of Weed*, Kruger National Park, South Africa, 2-7 March 2014 (**Appendix 4**).
5. Dhileepan, K., Nesar, S. and D.I. Rumiz. 2014. Biological control of bellyache bush (*Jatropha gossypifolia*): native range surveys in South America. Poster (**Appendix 5**).
6. Taylor, D.B.J. and Dhileepan, K. 2014. Growth of Queensland *Jatropha gossypifolia* biotypes under varying water regimes, pp. 333-336. In: Baker, M. (ed), *Proceedings of the 19th Australasian Weeds Conference*, Tasmanian Weed Society, Hobart, Tasmania, 1-4 September 2014 (**Appendix 6**).